REMARKS

Claims 66-113 have been withdrawn from consideration by the examiner. Claims 114-168 are pending in the present application and their rejection is discussed below.

The Invention

The present invention is drawn to methods of providing a therapeutic product to a mammal. The therapeutic product is produced by cells in which an endogenous gene encoding the protein is activated so that the protein is expressed from the genome of the cell. Activation in accordance with the invention is an *in vitro* process that involves targeted homologous recombination to place an exogenous regulatory sequence at a selected site within the genome in order to activate a selected endogenous gene. The construct used to accomplish this includes not only the exogenous regulatory sequence, but also (1) a targeting sequence, (2) an exon, (3) a splice-donor site, (4) an intron, and (5) a splice-acceptor site. The construct is introduced into a targeted cell *in vitro* by transfection (i.e., using a nonviral vector), and then homologously recombines with the cell's genome at a target site in the genome of the targeted cell. Transcription produces a transcript that contains sequence corresponding to the construct-derived exon, the construct-derived splice-donor site, the construct-derived intron, the construct-derived splice-acceptor site, and all of the exons of the endogenous gene, such that the RNA transcript encodes the therapeutic product. When this transcript undergoes splicing, the construct-derived splice-donor site interacts with the construct-derived splice-acceptor site. This results in the splicing out of the construct-derived intron, thereby producing an mRNA that is translated into a protein. Because this entire process is carried out *in vitro*, the resulting homologously recombined cells can be cultured. Those expressing the desired level of protein can be selected, expanded, and characterized prior to implantation of the cells into the mammal. This *ex vivo* method is therefore quite different from, and eliminates many of the uncertainties of, *in vivo* gene therapy techniques.

35 U.S.C. § 112, First Paragraph

Claims 114-168 have been rejected for alleged lack of enablement. Applicants and the Examiner agree that the invention is drawn to *ex vivo* methods (Office Action at page 1; as the Office Action does not have page numbers, for purposes of this Response, applicants have designated the first page containing the Examiner's remarks page 1). The rejection is generally based on allegations that undue experimentation is required to practice the claimed invention. Specifically, the Office Action raises concerns related to implantation of cells and adjustment of dosages (e.g., at pages 1 and 2), stability of expression of genes in *ex vivo* methods (e.g., at page 3), and an alleged lack of clinical evidence supporting the efficacy of gene therapy protocols (e.g., at page 5). Applicants respectfully disagree with the analyses provided in the Office Action and discuss these concerns below. Certain references are cited by the Examiner to support some of the assertions made in the Office Action. Applicants believe that the cited references do not support these assertions with respect to the claimed methods. The Examiner's concerns and the references are discussed individually.

Adjusting dosages

The Office Action does not accept applicants' arguments that determining the proper number of cells to implant in the methods of the invention would be a routine matter not requiring undue experimentation (Office Action at pages 1-2). In support of this part of the rejection, as well as in a number of other passages, the Examiner repeatedly makes statements that suggest a fundamental misapprehension of the present invention and how it differs from prior art gene therapy methods. For example, the Office Action states on page 2, first full paragraph, that "[I]t is clear that administering a protein composition to a patient is not analogous to administering a nucleic acid composition that encodes said protein. Neither the specification nor the art teaches how such a conversion calculation would be made." Again, on page 2, first paragraph, the Office Action states that "Applicants then argue that adjusting the dosage by adjusting the number of transfected cells administered is 'a simple and routine' task."

As pointed out in the previous response, the present invention does not involve the administration of a nucleic acid *per se* to a patient. Rather, the patient receives cells that produce the protein at a rate that has been carefully measured *in vitro* prior to implantation in the patient.

It is far more akin to implanting a device that secretes the protein, than to administering a nucleic acid that one hopes will be picked up and expressed by at least some of the patient's cells *in vivo*. In the present invention, once one determines *in vitro* how much protein is secreted per hour per cell, he can deliver protein into the patient at any desired rate by just implanting the proper number of cells. This is not a "conversion calculation." It is a simple arithmetic exercise: number of cells X amount of protein produced per cell per hour = dose of protein per hour. It is utterly simple and routine. The Examiner has not suggested any reason why it is "undue experimentation" to do this calculation, or to monitor the patient's condition, or to administer more cells if it is determined that the patient should receive a higher dosage of the protein, or to remove some cells if the dose proves to be too high. Whether administration of a given protein involves (1) injection of the protein, monitoring the patient's condition, and adjusting the dosage as needed (as in typical protein therapy), or (2) implantation of cells secreting the protein, monitoring the patient's condition, and adjusting the number of cells implanted as needed (as in the cell-based therapy of the invention), it is well within the skills of those who practice in this field. Other than concluding that the latter method is "trial-and-error experimentation performed directly on the patient" (without explaining why the former is not) (Office Action at page 2, first full paragraph), the Examiner has provided no reason why the latter "would have been deemed undue experimentation." He simply deems it so.

As the Examiner must realize, monitoring and adjusting dosage of drugs is routine clinical practice. See, for example, the attached section of The Merck Manual, Sixteenth Edition (Berkow, ed.; 1992; Merck Research Laboratories; Rahway, N.J.; Appendix A) that provides instruction for monitoring drug treatment and discusses adjustment of dosages indicated by such monitoring. Furthermore, applicants point out that methods of delivering a desired protein by implanting cells that secrete the protein, then adjusting the dosage, have been practiced by clinicians for many years. A standard treatment for hyperparathyroidism (overproduction of parathyroid hormone) involves surgical removal of the patient's parathyroid tissue and replacement of a fraction of the tissue back into the patient. As described in the following passage from Oxford Textbook of Surgery (1994, Morris and Malt, eds., Oxford University Press, New York), the patient's condition is monitored after treatment, and the number of

implanted cells is then increased or decreased as needed to provide the correct dosage of parathyroid hormone.

> An alternative strategy [to subtotal parathyroidectomy] ... is to remove all the parathyroid tissue from the neck, implant 50 to 60 mg. in the forearm, and to cryopreserve the rest. If the forearm transplant is too generous and hypercalcaemia persists or recurs then the surgeon has easy access to reduce the volume of transplanted tissue. If the patient becomes hypocalcaemic then the cryopreserved tissue is brought out of storage and some more is implanted.... [at pages 770, second column – 771, first column; see Appendix B].

Note that this parathyroid tissue procedure is apparently successfully practiced even without the precise characterization of the cells permitted by the present invention. Given that in the present invention, the cells can be cultured, cloned and characterized prior to implantation, one would expect that monitoring and adjusting dosage in the claimed methods would if anything be even more straightforward than in the parathyroid tissue example discussed above.

Clinical Success

The Office Action states (at the paragraph bridging pages 2 and 3) that "not one successful [gene therapy] protocol was known in the prior art...it is not clear how one of skill in the art would have been expected to proceed in practicing the claimed invention." Regardless of whether the statement that "not one successful protocol was known in the prior art" is true, applicants dispute its relevance to the present invention. Applicants' method is different from prior art methods of gene therapy, in large part because applicants' method employs an *ex vivo* method that permits selection of cells prior to implantation. Applicants demonstrated that *ex vivo* methods can work using an animal model in Example 9 of a related application, U.S.S.N. 07/787,840 ('840). For convenience, a copy of that Example 9 is attached as Appendix C. Applicants note that these are the *in vivo* results referred to in the June 4, 2001, response.

In Example 9 of '840, the present inventors (Treco, Selden, and Heartlein) introduced a human erythropoietin (EPO) gene into a mouse fibroblast. The cells were cultured and selected,

then implanted into mice. Expression of human EPO in the mice was observed for 12 months, the entire duration of the experiment. While that experiment involved expression of an exogenously introduced gene, rather than insertion of a new regulatory region that controls expression of an endogenous gene, it does provide evidence that, contrary to the assertions of the Office Action, expression in *ex vivo* therapies is not necessarily transient.

Applicants have also demonstrated the efficacy of *ex vivo* methods in clinical trials. Appendix D is a printout from the web site of the assignee of the present application, Transkaryotic Therapies, Inc. (TKT). It is an abstract presented at the 42nd American Society of Hematology Annual Meeting held on December 1-5, 2001. The abstract, entitled "Non-Viral Gene Transfer of Blood Coagulation Factor VIII in Patients with Severe Hemophilia A," reports results from animal studies as well as a phase I clinical study using cells transfected *ex vivo* with a gene encoding human factor VIII. The abstract relates that "durable expression of human factor VIII at levels that exceeded 5% of normal for greater than one year in mice following a single treatment [involving injection of *ex vivo* transfected and selected cells into the animals]." Furthermore, the abstract reports that three of six patients who were implanted with *ex vivo* transfected and selected cells "demonstrated repeated factor VIII activity levels above baseline, increasing to 1%-2% of normal, with a maximum of 4% measured in one patient. The increase of FVIII [factor VIII] activity coincided with decreased bleeding frequency or factor VIII use." These data illustrate the efficacy in general of non-viral *ex vivo* methods. The study reported in Appendix D involved the transfection of a gene into cells, followed by selection and implantation. This study was further reported in the New England Journal of Medicine (June 7, 2001) and is cited in Ferber (2001, Science 294:1638-1642, at page 1641, column 1; Appendix E) as an example of a non-viral strategy that produced therapeutic results in humans. There is no reason to believe that cells in which an endogenous gene is activated would not fare at least as well in similar *ex vivo* protocols. The data also demonstrate the effective use of an animal model in developing gene therapy protocols.

Persistent Expression

The Office Action states, as if it were fact, that "[t]ransfected cells quickly stop expression of transfected genes whether an *in vivo* or *ex vivo* method was employed" (Office

Action at page 3, first full paragraph), citing Anderson and Mountain to support this assertion. As discussed in detail below, applicants are aware of no published cases in which there was only transient expression of a transgene by implanted cells. Although long-lasting expression is acknowledged to be an issue for non-viral vectors used in *in vivo* gene therapy, applicants are unaware of any evidence that the same holds for *ex vivo* gene therapy. Even for *in vivo* gene therapy, transient expression is apparently not a consistent finding: in some cases (some of which are noted below), there is persistent expression. Furthermore, as pointed out by Ferber (2001, *supra*; Appendix E), transient expression in gene therapy is "in part because none of them stitch the useful gene into the genome of the host cell" (Ferber at page 1641, sentence bridging columns one and two). In contrast, the claimed method by its nature requires incorporation of the exogenous sequences into the genome. Finally, applicants note that the claims are not limited to methods involving long-term expression. Even transient expression can be useful, e.g., for eliciting an immune response or in treatment of tumors or myocardial ischemia.

Verma

The Office Action cites Verma "at page 240, paragraph bridging the left-hand and center columns, and bridging the center and left-hand columns" as "teach[ing] that transfected cells quickly stop expression of transfected genes, whether an in vivo or ex vivo method was employed." (Office Action at page 3, first full paragraph). The cited passage from Verma reports an experiment in which virus-infected cells ceased expressing Factor IX within five to seven days after implantation. However, the Examiner neglects to mention that Verma discloses a second experiment in which his infected cells continued to express Factor IX at "sustained and high levels" for more than two years after implantation in mice (page 240, col. 2, first full paragraph). Thus, if Verma's viral vector experiments have any relevance to the non-viral, gene activation methods of the invention, it is as a teaching that long-term, high level expression of implanted cells is achievable in some contexts. Beyond that, applicants believe there is little relevance to the present invention. Verma, after all, utilizes a viral vector that, if it integrates into the genome at all, does so at random sites. In contrast, the present invention utilizes a non-viral vector that can integrate at one and only one site: the desired target site. Furthermore, applicants have taught that cells expressing the desired level of protein should be selected,

cloned, and demonstrated to produce the protein consistently *in vitro* prior to implantation. Verma does not indicate that such steps were carried out, perhaps explaining why some experiments were less than successful. Finally, applicants have shown that these steps, when used with cells transfected with an exogenous gene homologously recombined at a selected target site, produce long-term, high level expression in both humans and animal models. The significance of Verma's results pales beside applicants' own highly relevant evidence.

Orkin

The Examiner continues to cite Orkin for its alleged teaching that persistence of expression is a problem in gene therapy (Office Action at page 3, first full paragraph). Applicants maintain that Orkin's teachings are not directed toward anything like the *ex vivo* methods of the present invention. The Examiner points to two passages in Orkin as supposedly discussing *ex vivo* methods. The second of the two (at the paragraph bridging the seventh and eighth pages of Orkin) does not (in applicants' copy, at least) even mention *ex vivo* therapy. It concerns "direct administration" of DNA *in vivo*. It therefore does not support the Examiner's argument. The other passage (at the sixth page, fifth full paragraph of Orkin) concerns use of retroviral vectors to introduce HIV genes into "target CD4 or precursor cells." Orkin does not even address the question of persistence of expression in this system, much less say it is a problem. In fact, one would not expect that persistent expression would be an issue for a vaccine, where all that is needed is temporary expression long enough to activate the immune system. The potential problems he does mention with respect to this HIV vaccine model (e.g., the type of cell to target) are simply not applicable to the present invention. In view of the above, it is curious that the Examiner would cite Orkin as supporting the present rejection. It appears to have no relevance whatsoever.

Anderson

The Office Action (at page 3, first full paragraph) cites Anderson at page 26, right-hand column, third full paragraph, as allegedly rebutting the contention that, in the Examiner's words, "merely implanting cells which express well when *in vitro* would have been a simple and predictable matter." Applicants see nothing in this paragraph, nor in those surrounding it, to

suggest that it was meant to apply to *ex vivo* methods. Keep in mind that the present methods permit culturing and selecting cells that continuously produce the protein of interest. This provides a powerful technique for ensuring the implanted cells produce the desired level of protein. As applicants have demonstrated (see above) *ex vivo* gene therapy does produce cells that continue to express the protein long after implantation.

Furthermore, applicants point out that Anderson references the well-known cases of two girls with adenosine deaminase deficiency who were implanted with gene-corrected autologous T lymphocytes approximately ten years ago. In addition to enjoying relatively normal health, the girls still had circulating engineered cells at least seven years after implantation (Anderson at page 29, first column, fifth full paragraph). Although these girls also receive other therapy for their disorder, the consensus is that the engineered cells are playing some role in their improved health. These results are consistent with not only clinical success of an *ex vivo* protocol, but also an apparent long-term effect.

Mountain

Like Anderson, Mountain is cited "in rebuttal to Applicants' contention that expression of genes transfected ex vivo remains stable in vivo." (Office Action at page 3, first full paragraph). The Examiner alleges that Mountain shows at Table 4 on page 22 "that non-viral methods of transfection in gene therapy, including ex vivo, do not lead to stable expression of the gene once implanted." (Office Action at page 3, first full paragraph). Applicants fail to see this teaching in Table 4, at least as applied to a method such as presently claimed. The cited Table does mention "very short duration of expression" as a disadvantage of the three non-viral techniques listed. However, Applicants disagree with the Office Action's assertion that one can derive from this Table a teaching that this disadvantage applies to both *in vivo* and *ex vivo* gene transfer, much less *ex vivo* gene transfer carried out as presently claimed. It is quite possible, and even likely, that the "short duration" problem is associated with any technique that produces primarily transient transfection, as would be true of most non-viral techniques (though not the methods of the invention). Worth noting is the fact that the Table does not cite "short duration" as a problem for those techniques typically characterized by integration into the cellular genome, i.e., the viral vector techniques. In fact, Mountain elsewhere cites publications reporting long

term *in vivo* expression by cells (a) infected *ex vivo* with a viral vector, then implanted (Miyoshi et al., 1999, Science 283:682-686; Appendix F) or (b) electroporated *in situ* using a non-viral vector (Nishi et al., 1997, Hum. Cell 10:81-86; Appendix G). The presently claimed methods employ homologous recombination, so necessarily involve chromosomal integration even though they are accomplished with non-viral vectors. Thus, the "short duration" problem mentioned in Table 4 of Mountain has no relevance to the present claims. **The most relevant evidence regarding duration of expression is that obtained by Applicants using an *ex vivo* transfection method with similarity to the presently claimed method, as discussed above.** As Applicants demonstrated, *ex vivo* homologous recombination, selection and expansion of cells results in cells that express the desired protein for extended periods, even after implantation into a subject. This evidence is far more relevant than anything the Examiner has cited to date.

Furthermore, Applicants query why the Examiner has based a lack of enablement rejection on this issue in the first place. Clearly cells that produce a desired protein for even a short time can be very useful for many purposes. The invention is not limited to production of proteins that will be needed indefinitely. Removal of the cells after their need has ended, or letting them gradually reduce production (if that indeed does ever happen), would be two ways to accomplish this.

CONCLUSION

Applicants submit that all claims are in condition for allowance, which action is respectfully requested. Enclosed is a $460 check for the Petition for Extension of Time fee. Please apply any other charges or credits to Deposit Account No. 06-1050, referencing attorney docket no 07236-013004.

Respectfully submitted,

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